

affinity reagents (e.g., capture ligands, redox mediators, particles, labels, etc.) may flow to initiate a desired electrochemical reaction. If desired, the additional washing channel and reagent channel may be printed in the manner described above. By using separate and distinct sample addition, washing, and reagent channels, the controlled and sequential delivery of different solutions may be provided.

[0047] Other techniques may also be employed in conjunction with, or in lieu of, the sample channel 14. In one embodiment, for example, a porous membrane or mesh (not shown) may be disposed on the substrate 40 and/or 80 that acts as a fluidic medium to transport the test sample to the detection working electrode 42. The pores of the membrane help guide the test sample to the detection working electrode 42 and may also help facilitate uniform mixing. In some cases, it may be desired that the “flow time” of the test sample through the membrane be long enough to promote uniform mixing and ensure that the analyte within the test sample has sufficient time to react with the desired reagents. For example, the time for the test sample to contact the detection working electrode 42 upon application may be at least about 1 minute, in some embodiments at least about 2 minutes, in some embodiments from about 3 to about 10 minutes, and in some embodiments, from about 4 to about 8 minutes. Such enhanced flow times are not only possible for test samples with high volumes, but also for test samples with low volumes. For example, test samples having a volume of less than about 100 microliters, in some embodiments from about 0.55 to about 50 microliters, and in some embodiments, from about 5 to about 35 microliters, may have an enhanced flow time. The ability to use such small test sample volumes is beneficial in that larger test volumes often increase background interference.

[0048] Without intending to be limited by theory, it is believed that the ability to achieve a long flow time for test samples with low volumes is a consequence of selectively controlling certain properties of the membrane, such as the shape or size of the membrane, the size of the pores, the material from which the membrane is formed (including its surface energy), etc. For example, the membrane may be selected to have any desired shape, such as a generally rectangular, square, circular, or any other regular or irregular shape. In some cases, one shape, such as a rectangular shape, may provide a longer flow time than another shape, such as a circular shape. Specifically, a generally rectangular membrane may have a long length (e.g., dimension that is substantially parallel to the flow of the test sample) and a small width (e.g., dimension that is substantially perpendicular to the flow of the test sample) to impart a slower flow rate. In some embodiments, for example, the width of a generally rectangular membrane may be from about 0.5 to about 10 millimeters, in some embodiments from about 1 to about 5 millimeters, and in some embodiments, from about 1 to about 3 millimeters. The length of such a membrane may be from about 1 to about 40 millimeters, in some embodiments from about 1 to about 20 millimeters, and in some embodiments, from about 1 to about 5 millimeters. The size of the pores may also affect the flow time of a test sample through the membrane. Specifically, smaller pore sizes often result in slower flow rates. In most embodiments, the pores of the membrane have an average size of from about 1 micron to about 50 microns, in some embodiments from about 5 microns to about 30 microns, and in some embodiments from about 5 microns to about 15 microns. If

desired, one or more dimensions of the membrane may be selected to correspond to a dimension of the detection working electrode 42. In this manner, most if not all of the test sample flowing through the membrane will contact a surface of the electrode 42, which alleviates possible background interference that might otherwise result due to the test sample flowing around the edges of the electrode 42. Alternatively, the electrode 42 and membrane may have different “actual” widths, but have substantially the same “effective” widths in that the portion of their widths exposed to the flow of the test sample is substantially the same. For instance, the width of the membrane may actually be larger than the width of the electrode 42. Nevertheless, the portion of the membrane’s width that is larger than that of the electrode 42 may be blocked to the flow of the test sample using, for instance, tape.

[0049] The materials used to form the membrane may also affect the flow time of the test sample. Some examples of suitable materials used to form the porous membrane may include, but are not limited to, natural, synthetic, or naturally occurring materials that are synthetically modified, such as polysaccharides (e.g., cellulose materials such as paper and cellulose derivatives, such as cellulose acetate and nitrocellulose); polyether sulfone; polyethylene; nylon; polyvinylidene fluoride (PVDF); polyester; polypropylene; silica; inorganic materials, such as deactivated alumina, diatomaceous earth,  $\text{MgSO}_4$ , or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon or rayon); porous gels, such as silica gel, agarose, dextran, and gelatin; polymeric films, such as polyacrylamide; and so forth. It should be understood that the term “nitrocellulose” refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, such as aliphatic carboxylic acids having from 1 to 7 carbon atoms. Without intending to be limited by theory, it is believed that the rate at which the test sample flows through the membrane may be greater for materials that are more hydrophilic in nature. Thus, for membranes of approximately the same pore size, shape, and dimensions, those made of nitrocellulose may result in a faster flow time than those made of polyvinylidene fluoride, which is somewhat less hydrophilic than nitrocellulose.

[0050] Referring again to FIG. 1, the substrate 80 also contains a sample pad 21 to which a test sample may be applied. Some suitable materials that may be used to form the sample pad 21 include, but are not limited to, nitrocellulose, cellulose, porous polyethylene pads, and glass fiber filter paper. If desired, the sample pad 21 may also contain one or more assay pretreatment reagents, either covalently or non-covalently attached thereto. In the illustrated embodiment, the test sample travels from the sample pad 21 to an optional conjugate pad 22 that is placed in communication with one end of the sample pad 21. The conjugate pad 22 is formed from a material through which the test sample is capable of passing. For example, in one embodiment, the conjugate pad 22 is formed from glass fibers. Although the analyte of interest may be inherently capable of undergoing the desired oxidation/reduction reactions because it contains a redox center, it may be desired, in other embodiments, to attach a redox label to the analyte. The redox label may be